

**United States Patent** [19]

Freytag et al.

[11] **Patent Number:** 4,517,303[45] **Date of Patent:** May 14, 1985[54] **SPECIFIC BINDING ASSAYS UTILIZING  
ANALYTE-CYTOLYSIN CONJUGATES**[75] **Inventors:** J. William Freytag, Wilmington;  
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Company, Wilmington, Del.[21] **Appl. No.:** 435,455[22] **Filed:** Oct. 20, 1982[51] **Int. Cl.<sup>3</sup>** ..... G01N 33/54; G01N 33/50;  
G01N 33/74[52] **U.S. Cl.** ..... 436/501; 435/4;  
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436/817; 436/827; 436/828; 436/829[58] **Field of Search** ..... 436/512, 532, 533, 534,  
436/808, 828, 829, 501, 520, 541, 803, 813, 815,  
817, 827; 435/4, 5, 7, 21, 36[56] **References Cited****U.S. PATENT DOCUMENTS**4,235,792 11/1980 Hsia et al. .... 260/403  
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3575 (1969).*Primary Examiner*—Sidney Marantz[57] **ABSTRACT**

A novel analyte-cytolysin conjugate and its use in a lipid vesicle mediated measurement process is described for a wide variety of analytes present at very low concentration. The method involves forming a reaction system consisting of analyte, analyte specific binding agent, analyte-cytolysin conjugate, and vesicles containing detectable marker material in such proportions that uncombined conjugate alters the permeability of the vesicles resulting in the release and quantitative detection of marker material which can be correlated with the amount of analyte initially present.

**40 Claims, 2 Drawing Figures**